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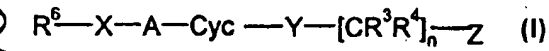
(54) Title: ALIPHATIC, CYCLIC AMINO CARBOXYLIC ACIDS AS INTEGRIN ANTAGONISTS

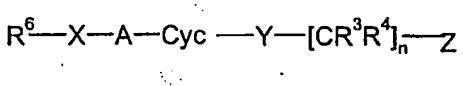


(I)

(57) Abstract: The present invention relates to compounds of the general formula (I), (I)processes for their preparation, pharmaceutical compositions containing them as well as their use for the production of pharmaceutical compositions for the treatment of inflammatory diseases.

Aliphatic, cyclic amino carboxylic acids as integrin antagonists

The present invention relates to compounds of formula (I),



$$R^6—X—A—Cyc—Y—[CR^3R^4]_n—Z \qquad (I)$$

their preparation and use as pharmaceutical compositions as integrin antagonists, especially as $\alpha_4\beta_1$ and/or $\alpha_4\beta_7$ and/or $\alpha_9\beta_1$ integrin antagonists and in particular for the production of pharmaceutical compositions suitable for the inhibition or the prevention of cell adhesion and cell-adhesion mediated disorders. Examples are the treatment and the prophylaxis of atherosclerosis, asthma, chronic obstructive pulmonary disease (COPD), allergies, diabetes, inflammatory bowel disease, multiple sclerosis, myocardial ischemia, rheumatoid arthritis, transplant rejection and other inflammatory, autoimmune and immune disorders.

Adhesive interactions between the leukocytes and endothelial cells play a critical role in leukocyte trafficking to sites of inflammation. These events are essential for normal host defense against pathogens and repair of tissue damage, but can also contribute to the pathology of a variety of inflammatory and autoimmune disorders. Indeed, eosinophil and T cell infiltration into the tissue is known as a cardinal feature of allergic inflammation such as asthma.

The interaction of circulating leukocytes with adhesion molecules on the luminal surface of blood vessels appears to modulate leukocyte transmigration. These vascular cell adhesion molecules arrest circulating leukocytes, thereby serving as the first step in their recruitment to infected or inflamed tissue sites. Subsequently, the leukocytes reaching the extravascular space interact with connective tissue cells such as fibroblasts as well as extracellular matrix proteins such as fibronectin, laminin, and collagen. Adhesion molecules on the leukocytes and on the vascular endothelium are hence essential to leukocyte migration and attractive therapeutic targets for intervention in many inflammatory disorders.

Leukocyte recruitment to sites of inflammation occurs in a stepwise fashion beginning with leukocyte tethering to the endothelial cells lining the blood vessels. This is followed by leukocyte rolling, activation, firm adhesion, and transmigration. A number of cell adhesion molecules involved in those four recruitment steps have been identified and characterized to date. Among them, the interaction between vascular cell adhesion molecule 1 (VCAM-1) and very late antigen 4 (VLA-4, $\alpha_4\beta_1$ integrin), as well as the interaction between mucosal addressin cell adhesion molecule 1 (MAdCAM-1) and $\alpha_4\beta_7$ integrin, has been shown to mediate the tethering, rolling, and adhesion of lymphocytes and eosinophils, but not neutrophils, to endothelial cells under a physiologic flow condition. This suggests that the VCAM-1 / VLA-4 and/or MAdCAM-1 / $\alpha_4\beta_7$ integrin mediated interactions could predominantly mediate a selective recruitment of leukocyte subpopulations *in vivo*. The inhibition of this interaction is a point of departure for therapeutic intervention (A. J. Wardlaw, *J. Allergy Clin. Immunol.* 1999, 104, 917-26).

VCAM-1 is a member of immunoglobulin (Ig) superfamily and is one of the key regulators of leukocyte trafficking to sites of inflammation. VCAM-1, along with intracellular adhesion molecule 1 (ICAM-1) and E-selectin, is expressed on inflamed endothelium activated by such cytokines as interleukin 1 (IL-1) and tumor necrosis factor α (TNF-α), as well as by lipopolysaccharide (LPS), via nuclear factor κB (NF-κB) dependent pathway. However, these molecules are not expressed on resting endothelium. Cell adhesion mediated by VCAM-1 may be involved in numerous physiological and pathological processes including myogenesis, hematopoiesis, inflammatory reactions, and the development of autoimmune disorders. Integrins VLA-4 and $\alpha_4\beta_7$ both function as leukocyte receptors for VCAM-1.

The integrin $\alpha_4\beta_1$ is a heterodimeric protein expressed in substantial levels on all circulating leukocytes except mature neutrophils. It regulates cell migration into tissues during inflammatory responses and normal lymphocyte trafficking. VLA-4 binds to different primary sequence determinants, such as a QIDSP motif of VCAM-

1 and an ILDVP sequence of the major cell type-specific adhesion site of the alternatively spliced type III connecting segment domain (CS-1) of fibronectin.

In vivo studies with neutralizing monoclonal antibodies and inhibitor peptides have demonstrated a critical role for α_4 integrins interaction in leukocyte-mediated inflammation. Blocking of VLA-4/ligand interactions, thus, holds promise for therapeutic intervention in a variety of inflammatory, autoimmune and immune diseases (Zimmerman, C.; *Exp. Opin. Ther. Patents* 1999, *9*, 129-133).

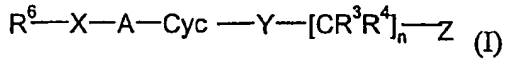
Furthermore, compounds containing a bisarylurea moiety as a substituent were disclosed as $\alpha_4\beta_1$ integrin receptor antagonists: WO 96/22966, WO 97/03094, WO 99/33789, WO 99/37605. However, no aminocycloalkyl carboxylic acids or homologues thereof or heterocyclics analogues thereof with $\alpha_4\beta_1$ integrin receptor antagonists activity have been described.

None of these compounds have been described in relation to the inhibition or the prevention of cell adhesion and cell-adhesion mediated disorders.

Further to their $\alpha_4\beta_1$ integrin antagonistic activity, the compounds of the present invention may also be used as $\alpha_4\beta_7$ or $\alpha_9\beta_1$ integrin antagonists.

An object of the present invention is to provide new, alternative, aminobenzoic acids or aminocycloalkylcarboxylic acids or homologues thereof or heterocyclic analogues thereof derived integrin antagonists for the treatment of inflammatory, autoimmune and immune diseases.

The present invention therefore relates to compounds of the general formula (I):



$$R^6—X—A—Cyc—Y—[CR^3R^4]_n—Z \quad (I)$$

wherein

Cyc represents a 5- or 6-membered carbocycle,
which can optionally be substituted with up to two residues R^{cyc},

wherein the residues R^{cyc} can independently be selected from the group consisting of halogen, trifluoromethyl, amino, nitro and cyano

A represents an amide moiety of the structure

$-NR^{A-1}C(O)-$ or $-C(O)NR^{A-1}-$,

wherein R^{A-1} represents hydrogen or C_1-C_{10} alkyl,

Z represents $-C(O)OR^{Z-1}$, $-C(O)NR^{Z-2}R^{Z-3}$, $-SO_2NR^{Z-2}R^{Z-3}$, $-SO(OR^{Z-1})$, $-SO_2(OR^{Z-1})$, $-P(O)R^{Z-1}(OR^{Z-3})$ or $-PO(OR^{Z-1})(OR^{Z-3})$,

wherein R^{Z-2} is hydrogen, C_1-C_4 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_6 or C_{10} aryl, $-C(O)R^{Z-4}$ or $-SO_2R^{Z-4}$,

wherein R^{Z-4} is C_1-C_4 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_6 or C_{10} aryl,

R^{Z-1} and R^{Z-3} are independently selected from the group hydrogen, C_1-C_4 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_6 or C_{10} aryl or benzyl,

wherein R^{Z-1} and R^{Z-3} can optionally be substituted by 1 to 3 substituents selected from the group C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, nitro, cyano,

R^3 represents OR^{3-1}, $NR^{3-2}R^{3-3}$,

wherein R^{3-1} represents hydrogen or C_1-C_4 alkyl, and
R^{3-2} and R^{3-3} are independently selected from the group hydrogen, C_1-C_4 alkyl and acyl,

or

R^3 represents phenyl, benzyl, benzyloxy or phenoxy, thiophenyl, C_1-C_4 alkyl, C_3-C_6 cycloalkyl, halogen, trifluoromethyl, nitro or cyano,

wherein phenyl, benzyl, benzyloxy or phenoxy, thiophenyl and C_1-C_4 alkyl can optionally be substituted with 0 to 2 substituents independently selected from the group group C_1-C_4 alkyl, C_3-C_6 cycloalkyl, C_1-C_4 alkoxy, halogen, nitro, cyano, carboxy, trifluormethoxy, $-NR^{3-4}R^{3-5}$,

wherein R^{3-4} and R^{3-5} are independently selected from the group hydrogen, C_1-C_4 alkyl and acyl,

R^4 represents OR^{4-1}, $NR^{4-2}R^{4-3}$,

wherein R^{4-1} represents hydrogen or C_1-C_4 alkyl, and
R^{4-2} and R^{4-3} are independently selected from the group hydrogen, C_1-C_4 alkyl and acyl,

or

R^4 represents phenyl, benzyl, benzyloxy or phenoxy, thiophenyl, C_1-C_4 alkyl, C_3-C_6 cycloalkyl, halogen, trifluoromethyl, nitro or cyano,

wherein phenyl, benzyl, benzyloxy or phenoxy, thiophenyl and C_1-C_4 alkyl can optionally be substituted with 0 to 2 substituents independently selected from the group group C_1-C_4 alkyl, C_3-C_6 cycloalkyl, C_1-C_4 alkoxy, halogen, nitro, cyano, carboxy, trifluormethoxy, $-NR^{4-4}R^{4-5}$,

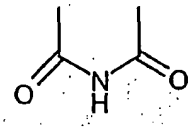
wherein R^{4-4} and R^{4-5} are independently selected from the group hydrogen, C_1-C_4 alkyl and acyl,

or R^3 and R^4 together with the carbon atom to which they are attached form a 5- to 7-membered ring, which can contain up to three heteroatoms selected from the group N, O and S,

R^6 represents phenyl or a 5- to 6-membered aromatic heterocyclic residue containing up to 3 heteroatoms independently selected from the group oxygen, nitrogen and sulfur,

which is substituted by $-NR^{6-2}C(O)NR^{6-3}R^{6-4}$ and can furthermore optionally be substituted by halogen,

wherein R^{6-2} and R^{6-3} are independently selected from the group hydrogen or C_1-C_4 alkyl, or together form a group



and wherein R^{6-4} represents phenyl,

wherein R^{6-4} can optionally be substituted by 1-2 substituents selected from the group C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, nitro, trifluoromethyl, trifluoromethoxy or cyano,

n represents an integer 2, 3 or 4,

X represents bond or $-CR^{X-1}R^{X-2}-$,

wherein R^{X-1} and R^{X-2} can be independently selected from the group hydrogen, C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl,

Y represents an amide moiety of the structure

$-NR^{Y-1}C(O)-$ or $-C(O)NR^{Y-1}-$,

wherein R^{Y-1} represents hydrogen or C_1-C_4 alkyl,

and pharmaceutically acceptable salts thereof.

In a preferred embodiment, the present invention relates to compounds of general formula (I), wherein Cyc represents a 5- membered carbocycle.

In another preferred embodiment, the present invention relates to compounds of general formula (I), wherein the moiety A-Cyc-Y represents a γ–amino acid.

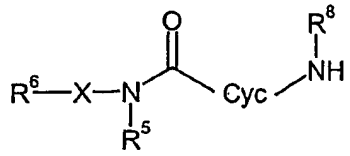
In another preferred embodiment, the present invention relates to compounds of general formula (I), wherein R^{1-1} represents a bond and Z represents $COOR^{Z-1}$, wherein R^{Z-1} has the meaning indicated above.

In another preferred embodiment, the present invention relates to compounds of general formula (I), wherein R^6 represents phenyl, which is substituted by $-NHC(O)NHR^{6-4}$, wherein R^{6-4} is substituted with methyl or trifluoromethoxy.

In another preferred embodiment, the present invention relates to compounds of general formula (I), wherein n is 3.

In another preferred embodiment, the present invention relates to compounds of general formula (I), wherein X represents bond.

A process for preparation of compounds of general formula (I) has also been found, which comprises reaction of compounds of general formula (I')

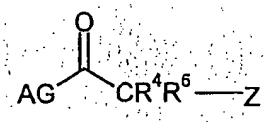


(I')

wherein

Cyc, X, R^5, R^6 and R^8 have the abovementioned meaning,

with compounds of the general formula (I'')



(I'')

wherein

R^4, R^5 and Z have the abovementioned meaning and AG represents an activating group,

in inert solvents.

In the context of the present invention alkyl stands for a straight-chain or branched alkyl residue, such as methyl, ethyl, n-propyl, iso-propyl, n-pentyl. If not stated otherwise, preferred is C_1-C_{10} alkyl, very preferred is C_1-C_6 alkyl.

Alkenyl and alkinyl stand for straight-chain or branched residues containing one or more double or triple bonds, e.g. vinyl, allyl, isopropinyl, ethinyl. If not stated otherwise, preferred is C_1-C_{10} alkenyl or alkinyl, very preferred is C_1-C_6 alkenyl or alkinyl.

Cycloalkyl stands for a cyclic alkyl group such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl. Preferred is C_3-C_7 cycloalkyl.

Halogen in the context of the present invention stands for fluorine, chlorine, bromine or iodine. If not specified otherwise, chlorine or fluorine are preferred.

Carbocycle stands for a ring consisting of carbon atoms.

Heteroaryl stands for a monocyclic heteroaromatic system containing 4 to 9 ring atoms, which can be attached via a carbon atom or eventually via a nitrogen atom within the ring, for example, furan-2-yl, furan-3-yl, pyrrol-1-yl, pyrrol-2-yl, pyrrol-3-yl, thienyl, thiazolyl, oxazolyl, imidazolyl, triazolyl, tetrazolyl, pyridyl, pyrimidyl or pyridazinyl.

A saturated or unsaturated heterocyclic residue stands for a heterocyclic system containing 4 to 9 ring atoms, which can contain one or more double bonds and which can be attached via a ring carbon atom or eventually via a nitrogen atom, e.g. tetrahydrofur-2-yl, pyrrolidine-1-yl, piperidine-1-yl, piperidine-2-yl, , piperidine-3-yl, piperidine-4-yl, piperazine-1-yl, piperazine-2-yl morpholine-1-yl, 1,4-diazepine-1-yl or 1,4-dihydropyridine-1-yl.

If not specified otherwise, in the context of the present invention heteroatom stands preferably for O, S, N or P.

Surprisingly, the compounds of the present invention show good integrin antagonistic activity. They are therefore suitable especially as $\alpha_4\beta_1$ and/or $\alpha_4\beta_7$ and/or $\alpha_9\beta_1$ integrin antagonists and in particular for the production of pharmaceutical compositions for the inhibition or the prevention of cell adhesion and cell-adhesion mediated disorders. Examples are the treatment and the prophylaxis of atherosclerosis, asthma, chronic obstructive pulmonary disease (COPD), allergies, diabetes, inflammatory bowel disease, multiple sclerosis, myocardial ischemia, rheumatoid arthritis, transplant rejection and other inflammatory, autoimmune and immune disorders.

The integrin antagonists of the invention are useful not only for treatment of the physiological conditions discussed above, but are also useful in such activities as purification of integrins and testing for activity.

For the treatment of the above-mentioned diseases, the compounds according to the invention can exhibit non-systemic or systemic activity, wherein the latter is preferred. To obtain systemic activity the active compounds can be administered, among other things, orally or parenterally, wherein oral administration is preferred.

For parenteral administration, forms of administration to the mucous membranes (i.e. buccal, lingual, sublingual, rectal, nasal, pulmonary, conjunctival or intravaginal) or into the interior of the body are particularly suitable. Administration can be carried out by avoiding absorption (i.e. intracardiac, intra-arterial, intravenous, intraspinal or intralumbar administration) or by including absorption (i.e. intracutaneous, subcutaneous, percutaneous, intramuscular or intraperitoneal administration).

For the above purpose the active compounds can be administered per se or in administration forms.

Suitable administration forms for oral administration are, inter alia, normal and enteric-coated tablets, capsules, coated tablets, pills, granules, pellets, powders, solid and liquid aerosols, syrups, emulsions, suspensions and solutions. Suitable administration forms for parenteral administration are injection and infusion solutions.

The active compound can be present in the administration forms in concentrations of from 0.001 - 100 % by weight; preferably the concentration of the active compound should be 0.5 - 90% by weight, i.e. quantities which are sufficient to allow the specified range of dosage.

The active compounds can be converted in the known manner into the abovementioned administration forms using inert non-toxic pharmaceutically suitable auxiliaries, such as for example excipients, solvents, vehicles, emulsifiers and/or dispersants.

The following auxiliaries can be mentioned as examples: water, solid excipients such as ground natural or synthetic minerals (e.g. talcum or silicates), sugar (e.g. lactose), non-toxic organic solvents such as paraffins, vegetable oils (e.g. sesame oil), alcohols (e.g. ethanol, glycerol), glycols (e.g. polyethylene glycol), emulsifying agents, dispersants (e.g. polyvinylpyrrolidone) and lubricants (e.g. magnesium sulphate).

In the case of oral administration tablets can of course also contain additives such as sodium citrate as well as additives such as starch, gelatin and the like. Flavour enhancers or colorants can also be added to aqueous preparations for oral administration.

For the obtainment of effective results in the case of parenteral administration it has generally proven advantageous to administer quantities of about 0.001 to 100 mg/kg, preferably about 0.01 to 1 mg/kg of body weight. In the case of oral administration

the quantity is about 0.01 to 100 mg/kg, preferably about 0.1 to 10 mg/kg of body weight.

It may nevertheless be necessary to use quantities other than those mentioned above, depending on the body weight concerned, the method of administration, the individual response to the active compound, the type of preparation and the time or interval of administration.

Suitable pharmaceutically acceptable salts of the compounds of the present invention that contain an acidic moiety include addition salts formed with organic or inorganic bases. The salt forming ion derived from such bases can be metal ions, e.g., aluminum, alkali metal ions, such as sodium of potassium, alkaline earth metal ions such as calcium or magnesium, or an amine salt ion, of which a number are known for this purpose. Examples include ammonium salts, arylalkylamines such as dibenzylamine and *N,N*-dibenzylethylenediamine, lower alkylamines such as methylamine, *t*-butylamine, procaine, lower alkylpiperidines such as *N*-ethylpiperidine, cycloalkylamines such as cyclohexylamine or dicyclohexylamine, 1-adamantylamine, benzathine, or salts derived from amino acids like arginine, lysine or the like. The physiologically acceptable salts such as the sodium or potassium salts and the amino acid salts can be used medicinally as described below and are preferred.

Suitable pharmaceutically acceptable salts of the compounds of the present invention that contain a basic moiety include addition salts formed with organic or inorganic acids. The salt forming ion derived from such acids can be halide ions or ions of natural or unnatural carboxylic or sulfonic acids, of which a number are known for this purpose. Examples include chlorides, acetates, trifluoroacetates, tartrates, or salts derived from amino acids like glycine or the like. The physiologically acceptable salts such as the chloride salts, the trifluoroacetic acid salts and the amino acid salts can be used medicinally as described below and are preferred.

These and other salts which are not necessarily physiologically acceptable are useful in isolating or purifying a product acceptable for the purposes described below.

The salts are produced by reacting the acid form of the invention compound with an equivalent of the base supplying the desired basic ion or the basic form of the invention compound with an equivalent of the acid supplying the desired acid ion in a medium in which the salt precipitates or in aqueous medium and then lyophilizing. The free acid or basic form of the invention compounds can be obtained from the salt by conventional neutralization techniques, e.g., with potassium bisulfate, hydro-chloric acid, sodium hydroxide, sodium bicarbonate, etc.

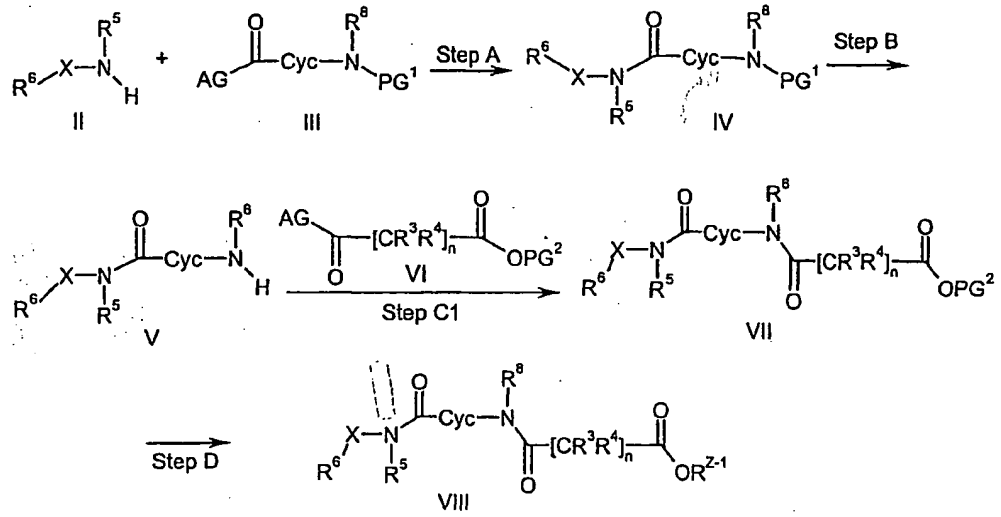
The compounds according to the invention can form non covalent addition compounds such as adducts or inclusion compounds like hydrates or clathrates. This is known to the artisan and such compounds are also object of the present invention.

The compounds according to the invention can exist in different stereoisomeric forms, which relate to each other in an enantiomeric way (image and mirror image) or in a diastereomeric way (image different from mirror image). The invention relates to the enantiomers and the diastereomers as well as their mixtures. They can be separated according to customary methods.

The compounds according to the invention can exist in tautomeric forms. This is known to the artisan and such compounds are also object of the present invention.

General compound synthesis

The synthesis of compounds according to the general formula (I) can be illustrated by the following scheme 1:



Scheme 1

By coupling of the amines (II) with the carboxylic acids or activated derivatives (III), followed by removal of the protecting group PG^1 the amides (V) can be obtained. Coupling with the carboxylic acids or activated derivatives (VI), and, if necessary, followed by removal of the protecting group PG^2, affords carboxylic acids of type (VIII).

In the above scheme AG stands for hydroxyl or a suitable activating group forming an activated carboxylic acid derivative. Activated carboxylic acids derivatives of this type are known to the person skilled in the art and are described in detail in standard textbooks such as, for example in (i) Houben-Weyl, Methoden der organischen Chemie [Methods of Organic Chemistry], Georg Thieme Verlag, Stuttgart or (ii) Comprehensive Organic Synthesis, Ed. B. M. Trost, Pergamon Press, Oxford, 1991. The carboxylic acid is preferably activated symmetrical anhydride or as mixed anhydride, such as, for example, AG = *iso*-butyl-carbonate or by a coupling agents such as, for example dicyclohexylcarbodiimid (DCC), 1-ethyl-3-(3'-dimethylamino-

propyl)carbodiimide×HCl (EDCI), 2-(7-aza-3-oxido-1H-1,2,3-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate. Other activated carboxylic acid derivatives such as, for example symmetric anhydrides, halides, or activated esters e.g. succinyl, pentafluorophenyl or N-hydroxybenzotriazole esters may also be employed.

In the above scheme PG^1 stands for a suitable protecting group of the amino group that is stable under the respective reaction conditions. Protecting groups of this type are known to the person skilled in the art and are described in detail in T. W. Greene, P. G. Wuts, *Protective Groups in Organic Synthesis*, 3rd ed., John Wiley, New York, 1999. The amino group is preferably protected by carbamates, PG^1 being for example *tert*-butyloxycarbonyl (Boc), 9-fluorenylmethyloxycarbonyl (FMOC) or benzyloxy-carbonyl (Cbz- / Z-) or other oxycarbonyl derivatives.

In the above scheme PG^2 stands for a suitable protecting group of the carboxyl group or $COOPG^2$ stands for the carboxylic group attached to a polymeric resin suitable for solid phase synthesis. Protecting groups of this type are known to the person skilled in the art and are described in detail in T. W. Greene, P. G. Wuts, *Protective Groups in Organic Synthesis*, 3rd ed., John Wiley, New York, 1999. The carboxyl group is preferably esterified, PG^2 being C_{1-6}-alkyl such as, for example, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, pentyl, isopentyl, neopentyl, hexyl, a C_{3-7}-cycloalkyl such as, for example, cyclopropyl, cyclopropylmethyl, cyclobutyl, cyclopentyl, cyclohexyl, an aryl such as, for example, phenyl, benzyl, tolyl or a substituted derivative thereof.

Step A

Formation of the amides (IV) can take place by reacting an activated form of the respective carboxylic acid (III), such as an *iso*-butylcarbonate or N-hydroxybenzotriazole ester - with the desired amine (II) or an acceptable salt thereof.

iso-Butylcarbonates can be prepared *in situ* by reaction of the N-protected amino acid (III) with *iso*-butylchloroformate as described below. Activated derivatives of the acids (III) such as other anhydrides, halides, esters e.g. succinyl, N-hydroxybenzotriazole or pentafluorophenyl esters or activated carboxylic acids obtained by the reaction with coupling agents such as, for example dicyclohexylcarbodiimid (DCC), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide×HCl (EDCI), 2-(7-aza-3-oxido-1H-1,2,3-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate may also be employed.

1-Hydroxy-1H-benzotriazol ester of (III) can be prepared, for example, by the reaction of the 1-hydroxy-1H-benzotriazol with the carboxylic acids (III) in presence of an coupling agents such as, for example, dicyclohexylcarbodiimid (DCC), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide×HCl (EDCI), 2-(7-aza-3-oxido-1H-1,2,3-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate. Further activated derivatives of the acids (III) such as other anhydrides, halides, esters e.g. succinyl or pentafluorophenyl esters or activated carboxylic acids obtained by the reaction with coupling agents such as, for example dicyclohexylcarbodiimid (DCC), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide×HCl (EDCI), 2-(7-aza-3-oxido-1H-1,2,3-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate may also be employed.

For example, amides of type (IV) can be prepared as follows:

1) Mixed anhydride procedure

A solution of the carboxylic acid derivative (III) and of N-methylmorpholine in an inert solvent was cooled to -15°C and *iso*-butyl chloroformate was added and stirred at 0°C. The amine (II) in an inert solvent was added at -15°C. The solution was stirred at 0°C, and at r.t. and was evaporated. The residue was redissolved in ethyl acetate, washed with aqueous acid and base, dried and evaporated. If necessary the

product was purified by trituration or by flash-chromatography or used without further purification.

2) 1-Hydroxy-1H-benzotriazol ester procedure

A solution of carboxylic acid, 1-hydroxy-1H-benzotriazol (HOBt) and 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide×HCl (EDCI) in an inert solvent is stirred at r.t.. After addition of the amine and a non-nucleophilic base such as ethyldiisopropylamine or potassium carbonate stirring is continued at r.t. or elevated temperature. After evaporation, the residue was redissolved in ethyl acetate, washed with aqueous acid and base, dried and evaporated. If necessary the product was purified by trituration or by flash-chromatography or used without further purification.

The above reactions and their implementation are well known to the person skilled in the art and are described in detail in standard textbooks such as, for example, in (i) Houben-Weyl, Methoden der organischen Chemie [Methods of Organic Chemistry], Georg Thieme Verlag, Stuttgart or Stuttgart or (ii) Comprehensive Organic Synthesis, Ed. B. M. Trost, Pergamon Press, Oxford, 1991.

Compounds of general formula (II) are commercially available, known or can be prepared by customary methods starting from known carboxylic acid derivatives.

When more than one choice of reaction methods exist, the person skilled in the art is able to choose the appropriate pathway according to selectivity and possible use of protecting groups such as described in T. W. Greene, P. G. Wuts, *Protective Groups in Organic Synthesis*, 3rd ed., John Wiley, New York, 1999.

Step B

The removal of protecting group PG^1 can be performed, depending on the nature of PG^1, either by an acid such as trifluoroacetic acid for example in the case PG^1 is *tert*-butyloxycarbonyl (Boc), a base such as piperidine for example in the case PG^1 is 9-

fluorenylmethyloxycarbonyl (FMOC) or by catalytic hydrogenation for example in the case PG^1 is benzyloxycarbonyl (Cbz- / Z-).

Step C1

Formation of the amides (VII) can take place by reacting the respective carboxylic acids (VI) - activated by a coupling agent such as DCC and HOBt; EDCI and HOBt or HATU – or its symmetrical anhydride with the desired amines (V) or an acceptable salt thereof. Activated derivatives of the acids (VI) such as halides, and esters e.g. succinyl or pentafluorophenyl esters may also be employed.

For example, amides (VII) can be prepared as follows:
A solution of carboxylic acid, HOBt and EDCI in an inert solvent is stirred at r.t.. After addition of the amine and a non-nucleophilic base such as ethyldiisopropyl-amine stirring is continued at r.t. or elevated temperature. The reaction mixture is poured into water and worked up by standard procedures.

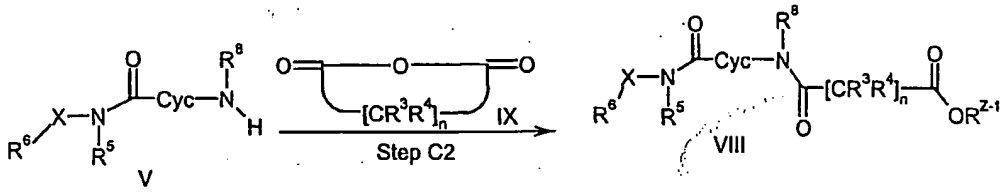
Compounds of general formula (VI) are commercially available, known or can be prepared by customary methods starting from known carboxylic acid derivatives.

Bisarylureas can be prepared by coupling of an amino phenyl acetic acid derivative and a phenylisocyanate. Cyanuric acid derivatives can be prepared by treatment of ureas with a-chlorocarbonyl isocyantes.

Step D

The removal of the protecting group PG^2 can be performed either by an acid such as trifluoroacetic acid or an base such as potassium hydroxide or lithium hydroxide, depending on the nature of PG^2. Reactions are carried out in aqueous, inert organic solvents such as alcohols e.g. methanol or ethanol, ethers e.g. tetrahydrofurane or dioxane or polar aprotic solvents e.g. dimethylformamide. If necessary, mixtures of the above solvents may be used.

An alternative synthesis of compounds according to the general formula (I), wherein n = 3,5,7,... represents substituted dicarboxylic acids, can be illustrated by the following scheme 2.



Scheme 2

The coupling of amides (V) with the carboxylic acid anhydrides (IX) affords carboxylic acids of type (VIII).

Step C2

Formation of the amides (VIII) can take place by reacting the respective carboxylic acid anhydrides (IX) with the desired amines (V) or an acceptable salt thereof.

The above reaction and their implementation are well known to the person skilled in the art and are described in detail in standard textbooks such as, for example, in (i) Houben-Weyl, Methoden der organischen Chemie [Methods of Organic Chemistry], Georg Thieme Verlag, Stuttgart or Stuttgart or (ii) Comprehensive Organic Synthesis, Ed. B. M. Trost, Pergamon Press, Oxford, 1991.

Compounds of general formula (IX) are commercially available, known or can be prepared by customary methods starting from known carboxylic acid derivatives.

Examples

Abbreviations

AcOH	acetic acid
Boc	*tert*-butyloxycarbonyl
DCC	dicyclohexylcarbodiimid
DCM	dichloromethane
DIPEA	diisopropylethylamine
EDCI	1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide×HCl
eq.	equivalents
EtOAc	ethyl acetate
FC	flash chromatography
GC	gas chromatography
HATU	2-(7-aza-3-oxido-1H-1,2,3-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HOBt	N-hydroxybenzotriazole monohydrate
HPLC	high performance liquid chromatography
ICAM-1	intracellular adhesion molecule 1
IL-1	interleukin 1
LPS	lipopolysaccharide
MAdCAM-1	mucosal addressin cell adhesion molecule 1
MeOH	methanol
MeCN	acetonitril
min.	minutes
M.p.	melting point
NF-κB	nuclear factor κB
NMR	nuclear magnetic resonance
n.d.	not determined
PE	light petroleum (b.p. 40-60 °C)
r.t.	room temperature
R_f	TLC: R_f value = distance spot traveled / distance solvent front traveled
TFA	trifluoroacetic acid

THF	tetrahydrofurane
TLC	thin layer chromatography
TNF-α	tumor necrosis factor α
t_R	retention time determined by HPLC
VCAM-1	vascular cell adhesion molecule 1
VLA-4	very late antigen 4 ($\alpha_4\beta_1$ integrin)

General remarks

In the examples below, all quantitative data, if not stated otherwise, relate to percentages by weight.

Flash chromatography was carried out on silica gel 60, 40–63µm (E. Merck, Darmstadt, Germany).

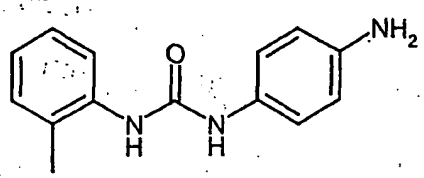
Thin layer chromatography was carried out, employing silica gel 60 F_{254} coated aluminum sheets (E. Merck, Darmstadt, Germany) with the mobile phase indicated.

Melting points were determined in open capillaries and are not corrected.

The mass determinations were carried out using the electron spray ionization (ESI) method employing loop injection or split injection via a HPLC system.

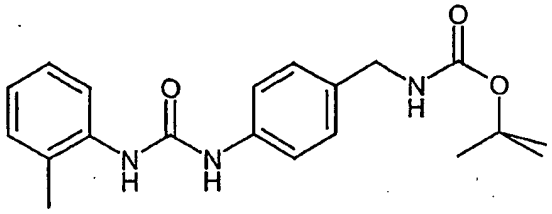
Precursor synthesis

Example I: N-(4-Aminophenyl)-N'-(2-methylphenyl)urea



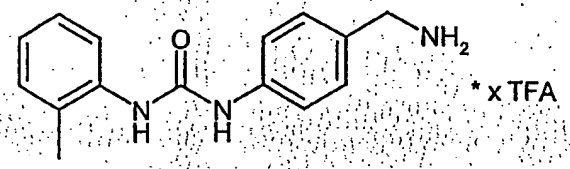
2-Methylphenylisocyanate (24.6 g, 184.9 mmol) was added dropwise at 0 °C to a solution of 1,4-diamino benzene (20.00 g, 184.9 mmol) in 1000 mL EtOAc. After stirring for 2 h at r.t. the product was collected by filtration (42.7 g, 177.0 mmol). M.p. >300 °C; TLC (PE/EtOAc 1/4) R_f 0.32; ^{1}H-NMR (400 MHz, D_6-DMSO) δ 2.10 (s, 3H); 4.76 (s, 2H); 6.59 (mc, 2H); 6.89 (mc, 1H); 7.07-7.15 (m, 4H); 7.73 (s, 1H); 7.85 (mc, 2H); 8.50 (s, 1H).

Example II: *tert*-Butyl 4-({[(2-methylphenyl)amino]carbonyl}amino)benzyl-carbamate



2-Methylphenylisocyanate (7.57 g, 59.83 mmol) was added dropwise at 0 °C to a solution of (4-amino-benzyl)-carbamic acid *tert*-butyl ester (13.30 g, 59.83 mmol; prepared analoguous to: Moloney, Gerard P.; Martin, Graeme R.; Mathews, Neil; Milne, Aynsley; Hobbs, Heather; et al. *J. Med. Chem.* **1999**, *42*, 2504 - 2526) in 120 mL DCM. The reaction was heated under reflux for 16 h, cooled to r.t. and the precipitated product was collected by filtration and dried in vacuum (19.20 g, 54.00 mmol). M.p. 200-202 °C; TLC (PE/EtOAc 1/1) R_f 0.65; ^{1}H NMR (400 MHz, D_6-DMSO) δ 1.39 (s, 9H); 2.24 (s, 3H); 4.06 (d, J=6 Hz, 2H); 6.93 (mc, 1H); 7.12-7.17 (m, 4); 7.32 (mc, 1H); 7.40 (mc, 2H); 7.85 (mc, 1H); 7.90 (s, 1H); 8.98 (s, 1H).

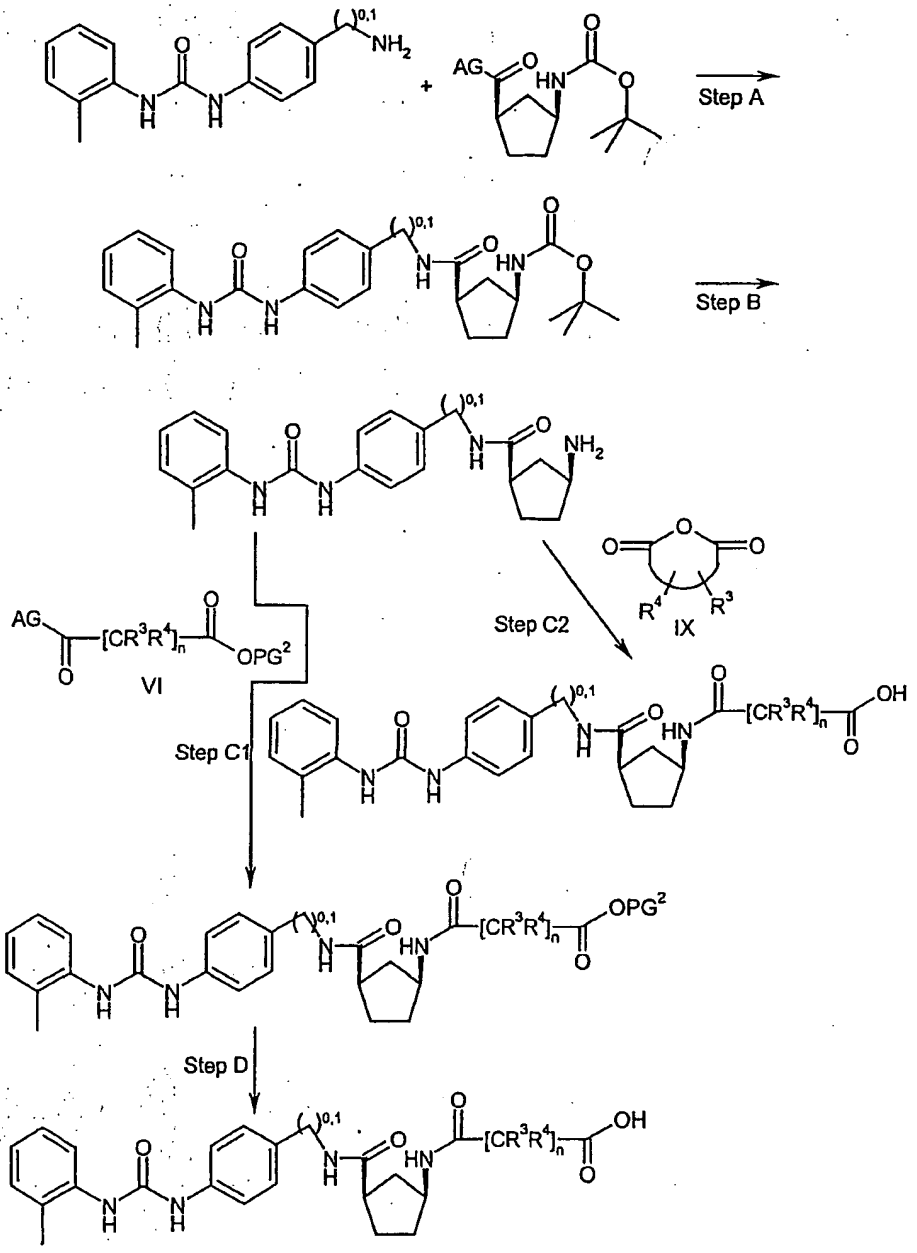
Example III: N-[4-(Aminomethyl)phenyl]-N'-(2-methylphenyl)urea



To a solution of *tert*-butyl 4-({[(2-methylphenyl)amino]carbonyl}amino)benzylcarbamate (2.00 g, 5.63 mmol) in CH_2Cl_2 (120 mL) TFA (36 mL) was added at 0 °C and stirred for 2 h at r.t.. The reaction mixture was evaporated and the product was collected (2.72 g, TFA salt). M.p. 142-143 °C; TLC (PE/EtOAc 3/2) R_f 0.14; ^1H NMR (400 MHz, D_6-DMSO) δ 2.24 (s, 3H); 3.97 (q, J=5 Hz, 2H); 6.96 (mc, 1H); 7.13-7.19 (m, 2); 7.36 (mc, 2H); 7.51 (mc, 2H); 7.81 (mc, 2H); 8.06 (s, 1H); 8.08 (s, 3H); 9.23 (s, 1H).

Compound synthesis

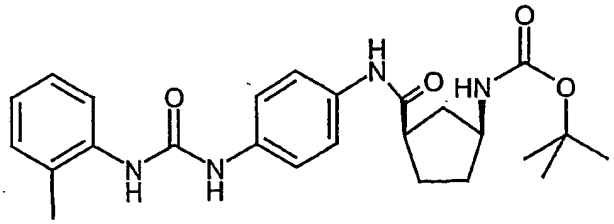
Examples 1-11 were prepared by the following general procedure.



Scheme 3

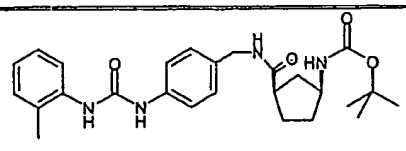
Step A:

Example IV: *tert*-Butyl-(1*S**,3*R**)-3-({[4-({[(2-methylphenyl)amino]carbonyl}-amino)phenyl]amino}carbonyl)cyclopentylcarbamate



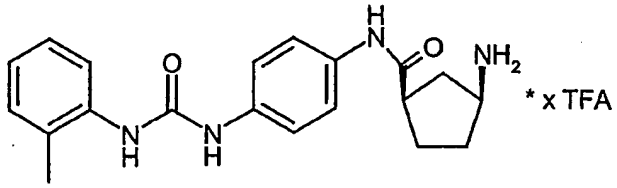
(1*S**,3*R**)-3-[(*tert*-Butoxycarbonyl)amino]cyclopentanecarboxylic acid (Lit.: Marco-Contelles, Jose; Bernabe, Manuel; *Tetrahedron Lett.* **1994**, *35*, 6361-6364) (2.00 g, 8.72 mmol) was dissolved in DMF (15 mL), HOBT (1.47 g, 9.60 mmol), EDCI (1.84 g, 9.60 mmol) and DIPEA (3.38 g, 26.17 mmol) were added at r.t. and stirred for 2 h. N-(4-Aminophenyl)-N'-(2-methylphenyl)urea (2.18 g, 9.60 mmol dissolved in 25 mL DMSO) was added and stirring was contined for 12 h. The reaction mixture was hydrolysed with ice, *tert*-butyl-(1*S**,3*R**)-3-({[4-({[(2-methylphenyl)amino]carbonyl}amino)phenyl]amino}carbonyl)cyclopentylcarbamate (3.32 g, 7.34 mmol) was collected by filtration, washed with water and isolated. M.p. 188-190 °C. ESI-MS: 453 $[M+H]^+$

Table 1: The following example was prepared according to the general procedure

No	Structure	Name	M.p. (°C)
V		*tert*-Butyl (1*S**,3*R**)-3-({[4-({[(2-methylphenyl)amino]-carbonyl}amino)benzyl]-amino}carbonyl)cyclopentyl carbamate	180-182

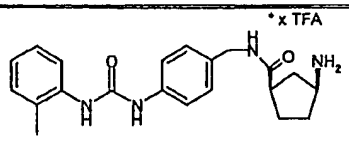
Step B:

Example VI: (1*R**,3*S**)-3-Amino-N-[4-({[(2-methylphenyl)amino]carbonyl}amino)-phenyl]cyclopentanecarboxamid



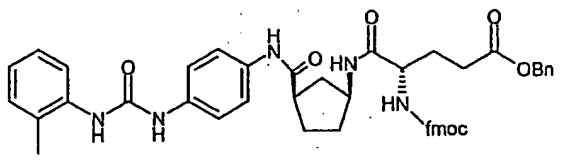
tert-Butyl-(1*S**,3*R**)-3-({[4-({[(2-methylphenyl)amino]carbonyl}amino)phenyl]amino}carbonyl)cyclopentylcarbamate was added to TFA (616 mL) at -5 °C and stirred for 0.75 h at r.t.. TFA was removed under vacuum, the residue was triturated with MTBE and DCM and dried, was collected (1*R**,3*S**)-3-amino-N-[4-({[(2-methyl-phenyl)amino]carbonyl}amino)phenyl]cyclopentanecarboxamid (23.87 g, TFA salt). ESI-MS: 353 $[M+H]^+$

Table 2: The following example was prepared according to the general procedure

No	Structure	Name
VII		(1*R**,3*S**)-3-Amino-N-[4-({[(2-methyl-phenyl)amino]carbonyl}amino)benzyl]-cyclopentanecarboxamide

Step C1:

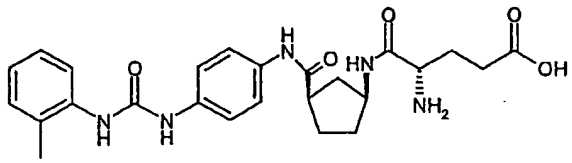
Example VIII: Benzyl N^2-[(9H-fluoren-9-ylmethoxy)carbonyl]- N^1-[(1*R**,3*S**)-3-({[4-({[(2-methylphenyl)amino]carbonyl}amino)phenyl]amino}carbonyl)cyclo-pentyl]-*L*-glutaminate



FMOC-L-Glutamic acid benzyl ester (359 mg, 0.78 mmol) was dissolved in DMF (4 mL), HOBT (144 mg, 0.94 mmol), EDCI (180 mg, 0.94 mmol) and DIPEA (240 mg, 1.88 mmol) were added. After stirring for 2 h at r.t., (1*R**,3*S**)-3-amino-N-[4-({[(2-methylphenyl)amino]carbonyl}amino)phenyl]cyclopentanecarboxamide (291 mg, TFA salt dissolved in 2 mL DMF) was added and stirring was continued for 12 h. The reaction mixture was hydrolysed with ice, N^1-[(1*S**,3*R**)-3-({[4-({[(2-methylphenyl)amino]carbonyl}amino)phenyl]amino}carbonyl)cyclopentyl]-α-glutamine (435 mg, 0.55 mmol) was collected by filtration, washed with water and isolated. ESI-MS: 838 $[M+HCOO]^+$

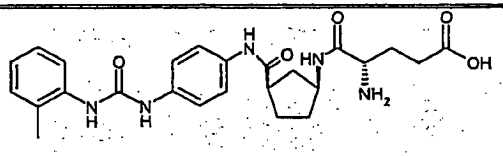
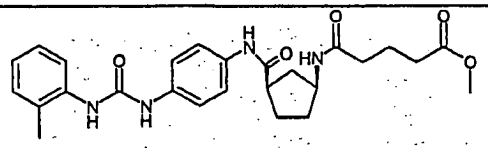
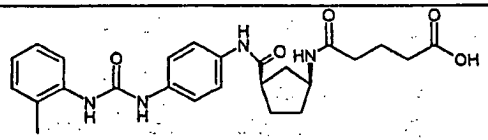
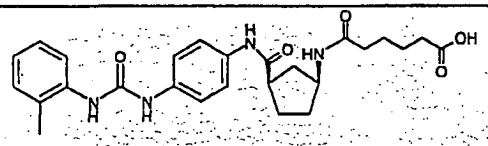
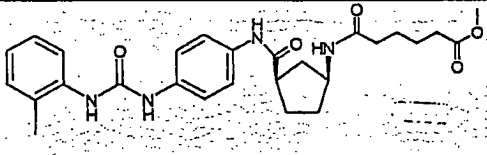
Step D:

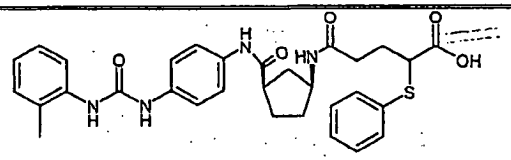
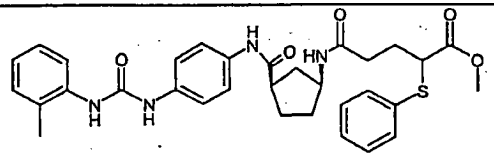
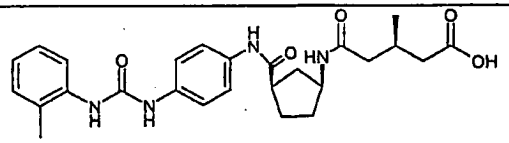
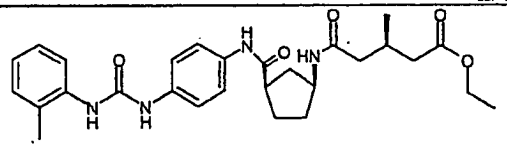
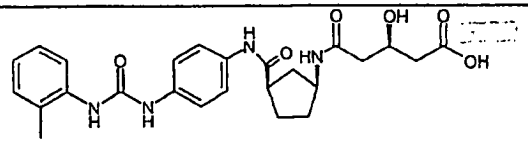
Example 1: N^1-[(1*S**,3*R**)-3-({[4-({[(2-Methylphenyl)amino]carbonyl}amino)-phenyl]amino}carbonyl)cyclopentyl]-α-glutamine

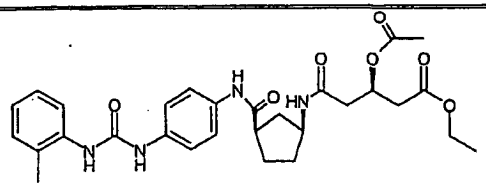
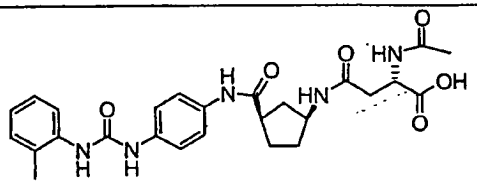
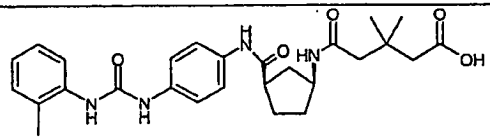
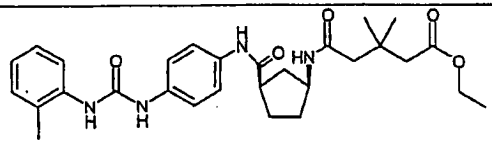
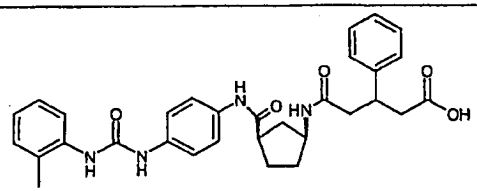


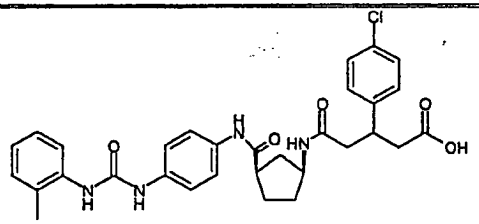
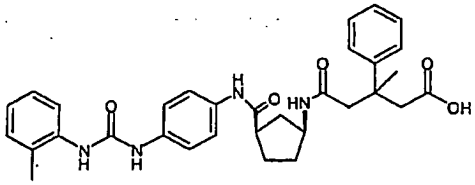
Benzyl N^2-[(9H-fluoren-9-ylmethoxy)carbonyl]- N^1-[(1*R**,3*S**)-3-({[4-({[(2-methyl-phenyl)amino]carbonyl}amino)phenyl]amino}carbonyl)cyclopentyl]-*L*-glutaminate (210 mg, 0.27 mmol) was dissolved in THF / water (1/1) and KOH (148 mg, 2.65 mmol) was added at r.t. The reaction mixture was stirred at 55 °C for 2 h. MTBE (5 mL) was added, the phases were separated, the aqueous phase was acidified (pH < 2) and extracted with EE (4*5 mL). The combined organic layers were dried, evaporated and N^1-[(1*S**,3*R**)-3-({[4-({[(2-Methylphenyl)amino]carbonyl}amino)phenyl]amino}carbonyl)cyclopentyl]-α-glutamine (31 mg, 0.06 mmol) was isolated as crystalline material. ESI-MS: 482 $[M+H]^+$

Table 3: The following examples were prepared according to the general procedure

No	Structure	Procedure	Name	ESI-MS	M.p. (°C)
1		C1	N^1-[(1S^*,3R^*)-3-({[4-({[(2-Methylphenyl)amino]carbonyl}amino)phenyl]amino}carbonyl)cyclopentyl]-α-glutamine	482 $[M+H]^+$	n.d.
2		C1	Methyl 5-{[(1S^*,3R^*)-3-({[4-({[(2-methylphenyl)-amino]carbonyl}amino)phenyl]amino}carbonyl)-cyclopentyl]amino}-5-oxopentanoate	481 $[M+H]^+$	n.d.
3		C1	5-{[(1S^*,3R^*)-3-({[4-({[(2-Methylphenyl)amino]carbonyl}amino)phenyl]amino}carbonyl)cyclopentyl]-amino}-5-oxopentanoic acid	467 $[M+H]^+$	260-264
4		C1	6-{[(1S^*,3R^*)-3-({[4-({[(2-Methylphenyl)amino]carbonyl}amino)phenyl]amino}carbonyl)cyclopentyl]-amino}-6-oxohexanoic acid	481.42 $[M+H]^+$	n.d.
5		C1	Methyl 6-{[(1S^*,3R^*)-3-({[4-({[(2-methylphenyl)-amino]carbonyl}amino)phenyl]amino}carbonyl)-cyclopentyl]amino}-6-oxohexanoate	495.4 $[M+H]^+$	n.d.

No	Structure	Procedure	Name	ESI-MS	M.p. (°C)
6		C1	5-{[(1*S**,3*R**)-3-({[4-({[(2-Methylphenyl)amino]carbonyl}amino)phenyl]amino}carbonyl)cyclopentyl]-amino}-5-oxo-2-(thiophenyl)pentanoic acid	575.44 $[M+H]^+$	n.d.
7		C1	Methyl 5-{[(1*S**,3*R**)-3-({[4-({[(2-methylphenyl)-amino]carbonyl}amino)phenyl]amino}carbonyl)-cyclopentyl]amino}-5-oxo-2-(thiophenyl)pentanoate	589.3 $[M+H]^+$	n.d.
8		C1	(3*R*)-3-Methyl-5-{[(1*S**,3*R**)-3-({[4-({[(2-methyl-phenyl)amino]carbonyl}amino)phenyl]amino}-carbonyl)cyclopentyl]amino}-5-oxopentanoic acid	481.41 $[M+H]^+$	n.d.
9			Ethyl (3*R*)-3-methyl-5-{[(1*S**,3*R**)-3-({[4-({[(2-methylphenyl)amino]carbonyl}amino)phenyl]amino}-carbonyl)cyclopentyl]amino}-5-oxopentanoate	509.3 $[M+H]^+$	n.d.
10		C1	(3*R*)-3-Hydroxy-5-{[(1*S**,3*R**)-3-({[4-({[(2-methyl-phenyl)amino]carbonyl}amino)phenyl]amino}-carbonyl)cyclopentyl]amino}-5-oxopentanoic acid	483.39 $[M+H]^+$	n.d.

No	Structure	Procedure	Name	ESI-MS	M.p. (°C)
11			Ethyl (3*R*)-3-(acetyloxy)-5-{[(1*S**,3*R**)-3-({[4-({[(2-methylphenyl)amino]carbonyl}amino)phenyl]-amino}carbonyl)cyclopentyl]amino}-5-oxopentanoate	553.4 $[M+H]^+$	n.d.
12		C1	N^2-Acetyl-N^4-[(1*S**,3*R**)-3-({[4-({[(2-methylphenyl)-amino]carbonyl}amino)phenyl]amino}carbonyl)-cyclopentyl]-*L*-asparagine	510.40 $[M+H]^+$	205-207
13		C1	3,3-Dimethyl-5-{[(1*S**,3*R**)-3-({[4-({[(2-methyl-phenyl)amino]carbonyl}amino)phenyl]amino}carb-onyl)cyclopentyl]amino}-5-oxopentanoic acid	495.44 $[M+H]^+$	222-223
14		C1	Methyl 3,3-dimethyl-5-{[(1*S**,3*R**)-3-({[4-({[(2-methylphenyl)amino]carbonyl}amino)phenyl]-amino}carbonyl)cyclopentyl]amino}-5-oxopentanoate	523.5 $[M+H]^+$	n.d.
15		C2	5-{[(1*S**,3*R**)-3-({[4-({[(2-Methylphenyl)amino]-carbonyl}amino)phenyl]amino}carbonyl)cyclo-pentyl]amino}-5-oxo-3-phenylpentanoic acid	543.5 $[M+H]^+$	223-225

No	Structure	Procedure	Name	ESI-MS	M.p. (°C)
16		C2	3-(4-Chlorophenyl)-5-{[(1*S**,3*R**)-3-({[4-({[(2-methylphenyl)-amino]carbonyl}amino)phenyl]amino}carb-onyl)cyclopentyl]amino}-5-oxopentanoic acid	577 $[M+H]^+$	243-247
17		C2	3-Methyl-5-{[(1*S**,3*R**)-3-({[4-({[(2-methylphenyl)-amino]carbonyl}amino)phenyl]amino}carbonyl)-cyclopentyl]amino}-5-oxo-3-phenylpentanoic acid	557.4 $[M+H]^+$	221-228

In vitro assay: adhesion of Ramos cells to immobilized VCAM-1 (domains 1-3)

Preparation of VCAM-1 (extracellular domains 1-3)

Complementary DNA (cDNA) encoding 7-domain form of VCAM-1 (GenBank accession #M60335) was obtained using Rapid-ScreenTM cDNA library panels (OriGene Technologies, Inc) at Takara Gene Analysis Center (Shiga, Japan). The primers used were 5'-CCA AGG CAG AGT ACG CAA AC-3' (sense) and 5'-TGG CAG GTA TTA TTA AGG AG-3' (antisense). PCR amplification of the 3-domain VCAM-1 cDNA was perform using *Pfu* DNA polymerase (Stratagene) with the following sets of primers: (U-VCAMd1-3) 5'-CCA TAT GGT ACC TGA TCA ATT TAA AAT CGA GAC CAC CCC AGA A-3'; (L-VCAMd1-3) 5'-CCA TAT AGC AAT CCT AGG TCC AGG GGA GAT CTC AAC AGT AAA-3'. PCR cycle was 94 °C for 45 sec, 55 °C for 45 sec, 72 °C for 2 min, repeating 15 cycles. After the purification of the PCR product, the fragment was digested with KpnI-AvrII. The digested fragment was ligated into pBluescript IISK(-) (Strategene), which was linearized by digesting with KpnI-XhoI. The ligation was followed by transformation to a Dam/Dcm methylase-free E. coli strain SCS110 (Strategene) to create the donor plasmid pHH7. To direct VCAM-1 molecule into the insect cell secretory pathway, the VCAM-1 coding sequence was fused to signal peptide sequence of honeybee melittin. The resulting melittin-VCAM fusion was placed in correct orientation to the baculovirus polyhedrin promoter. Baculovirus transfer vector containing first 3-domain form VCAM-1 (pH10) was constructed by ligation of 0.9 kb fragment from AvrII/Klenow/BclI digests of pH7 into SalI/Klenow/BamHI digests of pMelBacB (Invitrogen). Recombinant baculovirus was generated by using Bac-N-Blue™ Transfection kit (Invitrogen) according to the manufacture's instruction. The recombinant virus was amplified by infection to High-Five™ insect cells for 5 – 6 days, and virus titer was determined by plaque assay.

High-Five™ insect cells were pelleted in a 225 ml conical tube by centrifugation at 1000 rpm for 5 min. After discarding the supernatant, the pellet was resuspended in 1.5 x 10^9 pfu (MOI = 5) of high-titer virus solution, followed by incubation for 1.5

hours at room temperature. The cells were pelleted again and washed once in fresh Express Five™ serum free medium. The cells were pelleted again and finally, resuspended in 200 ml of fresh Express Five TM medium, transferred to a 1,000 ml shaker flask, and incubated in a shaker at 27 °C, 130 rpm, for 48 hours before the culture supernatant was collected. The purification of 3-domain form of VCAM-1 from the culture supernatant was performed by one-step anion exchange chromatography. Protein concentration was determined by using Coomassie protein assay reagent (Pierce) according to the manufacture's instruction.

Preparation of VCAM-1 coated microtiter plates

Recombinant human VCAM-1 (extracellular domains 1-3) was dissolved at 1.0 µg/ml in PBS. Each well of the microtiter plates (Nalge Nunc International, Fluoronunc Cert, 437958) was coated with 100 µl of substrate or for background control with buffer alone for 15 hours at 4 C. After discarding the substrate solution, the wells were blocked using 150 µl per well of block solution (Kirkegaard Perry Laboratories, 50-61-01) for 90 minutes. The plate was washed with wash buffer containing 24 mM Tris-HCl (pH 7.4), 137 mM NaCl, 27 mM KCl and 2 mM $MnCl_2$ just before addition of the assay.

In Vitro **Assay using Ramos cells**

Preparation of fluorescence labeled Ramos cells:

Ramos cells (American Type Culture Collection, Clone CRL-1596) were cultured in RPMI 1640 medium (Nikken Bio Medical Laboratory, CM1101) supplemented with 10% fetal bovine serum (Hyclone, A-1119-L), 100 U/ml penicilin (Gibco BRL, 15140-122) and 100 µg/ml streptomycin (Gibco BRL, 15140-122) in a humidified incubator at 37 °C with 5% CO_2.

Ramos cells were incubated with phosphate balanced solution (PBS, Nissui, 05913) containing 25 µM of 5(-and –6)-carboxyfluorescein diacetate, succinimidyle ester

(CFSE, Dojindo Laboratories, 345-06441) for 20 min at room temperature while gently swirling every 5 min. After centrifugation at 1000 rpm for 5 min, the cell pellet was resuspended with adhesion assay buffer at a cell density of 4 x 10^6 cells/ml. The adhesion assay buffer was composed of 24 mM Tris-HCl (pH 7.4), 137 mM NaCl, 27 mM KCl, 4 mM glucose, 0.1 % bovine serum albumin (BSA, Sigma, A9647) and 2 mM $MnCl_2$.

Assay procedure (Ramos cells)

The assay solution containing each test compounds or 5 µg/ml anti-CD49d monoclonal antibody (Immunotech, 0764) was transferred to the VCAM-1 coated plates. The final concentration of each test compounds was 5 µM, 10 µM or various concentrations ranging from 0.0001 µM to 10 µM using a standard 5-point serial dilution. The assay solution containing the labeled Ramos cells was transferred to the VCAM-1 coated plates at a cell density of 2 x 10^5 cells per well and incubated for 1 hour at 37 C. The non-adherent cells were removed by washing the plates 3 times with wash buffer. The adherent cells were broken by addition of 1 % Triton X-100 (Nacalai Tesque, 355-01). Released CFSC was quantified fluorescence measurement in a fluorometer (Wallac, ARVO 1420 multilabel counter).

The adhesion of Ramos cells to VCAM-1 was analyzed by percent binding calculated by the formula:

100 x (FTS – FBG) / (FTB – FBG) = % binding, where FTB is the total fluorescent intensity from VCAM-1 coated wells without test compound; FBG is the fluorescent intensity from wells with anti-CD49d monoclonal antibody and FTS is the fluorescent intensity from wells containing the test compound of this invention.

***In vitro* activity:**

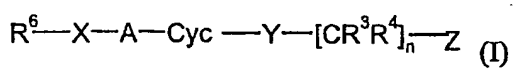
In the Ramos–VCAM-1 assay the observed IC_{50} value ranges are indicated in Table 4.

Table 4: A > 10 μM ≥ B ≥ 1 μM ≥ C

No	IC_{50}
1	A
3	B
4	A
6	A
8	B
10	A
12	B
13	B
15	C
16	C
17	C

Claims

1. Compounds of the general formula (I),



$$R^6—X—A—Cyc—Y—[CR^3R^4]_n—Z \quad (I)$$

wherein

Cyc represents a 5- or 6-membered carbocycle,
which can optionally be substituted with up to two residues R^{cyc},

wherein the residues R^{cyc} can independently be selected from the group consisting of halogen, trifluoromethyl, amino, nitro and cyano

A represents an amide moiety of the structure

$-NR^{A-1}C(O)-$ or $-C(O)NR^{A-1}-$,

wherein R^{A-1} represents hydrogen or C_1-C_{10} alkyl,

Z represents $-C(O)OR^{Z-1}$, $-C(O)NR^{Z-2}R^{Z-3}$, $-SO_2NR^{Z-2}R^{Z-3}$, $-SO(OR^{Z-1})$, $-SO_2(OR^{Z-1})$, $-P(O)R^{Z-1}(OR^{Z-3})$ or $-PO(OR^{Z-1})(OR^{Z-3})$,

wherein R^{Z-2} is hydrogen, C_1-C_4 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_6 or C_{10} aryl, $-C(O)R^{Z-4}$ or $-SO_2R^{Z-4}$,

wherein R^{Z-4} is C_1-C_4 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_6 or C_{10} aryl,

R^{Z-1} and R^{Z-3} are independently selected from the group hydrogen, C_1-C_4 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_3-C_6 cycloalkyl, C_6 or C_{10} aryl or benzyl,

wherein R^{Z-1} and R^{Z-3} can optionally be substituted by 1 to 3 substituents selected from the group C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, nitro, cyano,

R^3 represents OR^{3-1}, $NR^{3-2}R^{3-3}$,

wherein R^{3-1} represents hydrogen or C_1-C_4 alkyl, and
R^{3-2} and R^{3-3} are independently selected from the group hydrogen, C_1-C_4 alkyl and acyl,

or

R^3 represents phenyl, benzyl, benzyloxy or phenoxy, thiophenyl, C_1-C_4 alkyl, C_3-C_6 cycloalkyl, halogen, trifluoromethyl, nitro or cyano,

wherein phenyl, benzyl, benzyloxy or phenoxy, thiophenyl and C_1-C_4 alkyl can optionally be substituted with 0 to 2 substituents independently selected from the group group C_1-C_4 alkyl, C_3-C_6 cycloalkyl, C_1-C_4 alkoxy, halogen, nitro, cyano, carboxy, trifluormethoxy, $-NR^{3-4}R^{3-5}$,

wherein R^{3-4} and R^{3-5} are independently selected from the group hydrogen, C_1-C_4 alkyl and acyl,

R^4 represents OR^{4-1}, $NR^{4-2}R^{4-3}$,

wherein R^{4-1} represents hydrogen or C_1-C_4 alkyl, and

R^{4-2} and R^{4-3} are independently selected from the group hydrogen, C_1-C_4 alkyl and acyl,

or

R^4 represents phenyl, benzyl, benzyloxy or phenoxy, thiophenyl, C_1-C_4 alkyl, C_3-C_6 cycloalkyl, halogen, trifluoromethyl, nitro or cyano,

wherein phenyl, benzyl, benzyloxy or phenoxy, thiophenyl and C_1-C_4 alkyl can optionally be substituted with 0 to 2 substituents independently selected from the group group C_1-C_4 alkyl, C_3-C_6 cycloalkyl, C_1-C_4 alkoxy, halogen, nitro, cyano, carboxy, trifluormethoxy, $-NR^{4-4}R^{4-5}$,

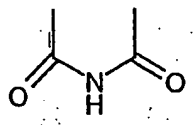
wherein R^{4-4} and R^{4-5} are independently selected from the group hydrogen, C_1-C_4 alkyl and acyl,

or R^3 and R^4 together with the carbon atom to which they are attached form a 5- to 7-membered ring, which can contain up to three heteroatoms selected from the group N, O and S,

R^6 represents phenyl or a 5- to 6-membered aromatic heterocyclic residue containing up to 3 heteroatoms independently selected from the group oxygen, nitrogen and sulfur,

which is substituted by $-NR^{6-2}C(O)NR^{6-3}R^{6-4}$ and can furthermore optionally be substituted by halogen,

wherein R^{6-2} and R^{6-3} are independently selected from the group hydrogen or C_1-C_4 alkyl, or together form a group



and wherein R^{6-4} represents phenyl,

wherein R^{6-4} can optionally be substituted by 1-2 substituents selected from the group C_1-C_4 alkyl, C_1-C_4 alkoxy, halogen, nitro, trifluoromethyl, trifluoromethoxy or cyano,

n represents an integer 2, 3 or 4,

X represents bond or $-CR^{X-1}R^{X-2}-$,

wherein R^{X-1} and R^{X-2} can be independently selected from the group hydrogen, C_1-C_4 alkyl, C_2 - C_4 alkenyl, C_2-C_4 alkynyl,

Y represents an amide moiety of the structure

$-NR^{Y-1}C(O)-$ or $-C(O)NR^{Y-1}-$,

wherein R^{Y-1} represents hydrogen or C_1-C_4 alkyl,

and pharmaceutically acceptable salts thereof.

2. Compounds of general formula (I) according to claim 1,

wherein Cyc represents a 5- membered carbocycle.

3. Compounds of general formula (I) according to claim 1 or 2,

wherein the moiety A-Cyc-Y represents a γ–amino acid.

4. Compounds of general formula (I) according to any one of claims 1 to 3,

wherein R^{1-1} represents a bond and Z represents $COOR^{Z-1}$,
wherein R^{Z-1} has the meaning indicated above.

5. Compounds of general formula (I) according to any one of claims 1 to 4,

wherein R^6 represents phenyl, which is substituted by $-NHC(O)NHR^{6-4}$,
wherein R^{6-4} is substituted with methyl or trifluoromethoxy.

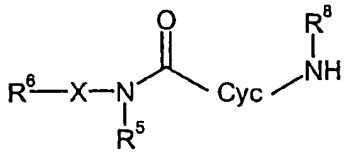
6. Compounds of general formula (I) according to any one of claims 1 to 5,

wherein n is 3.

7. Compounds of general formula (I) according to any one of claims 1 to 6,

wherein X represents bond.

8. A process for preparation of compounds of general formula (I) according to any one of claims 1 to 7, which comprises reaction of compounds of general formula (I')

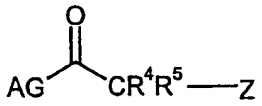


(I')

wherein

Cyc, X, R^5, R^6 and R^8 have the abovementioned meaning,

with compounds of the general formula (I'')



(I'')

wherein

R^4, R^5 and Z have the abovementioned meaning and AG represents an activating group,

in inert solvents.

9. The use of a compound according to any one of claims 1 to 7 in the manufacture of a medicament.

10. The use of a compound according to any one of claims 1 to 7 in the manufacture of a medicament for the treatment or the prevention of a condition mediated by integrins.

11. The use of a compound according to any one of claims 1 to 7 in the manufacture of a medicament for the treatment or the prevention of atherosclerosis, asthma, chronic obstructive pulmonary disease (COPD), allergies, diabetes, inflammatory bowel disease, multiple sclerosis, myocardial ischemia, rheumatoid arthritis, transplant rejection and other inflammatory, autoimmune and immune disorders.

12. Pharmaceutical composition, comprising compounds according to any one of claims 1 to 7 and a pharmaceutically acceptable carrier.